Esp

Week 6

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 SDS page for pSB1K3-T7-EB expression

Responsible

Shuangjia Xue and Oscar Frisell

Protocols used

SDS page

Modifications and comments to protocols

Sample preparation - Step 1:	Protein samples prepared on the 27th July
Gel preparation - Step 2:	12% acrylamide of the separating gel
Electrophoresis - Step 3:	100 V for 90 min

Results and Conclusions

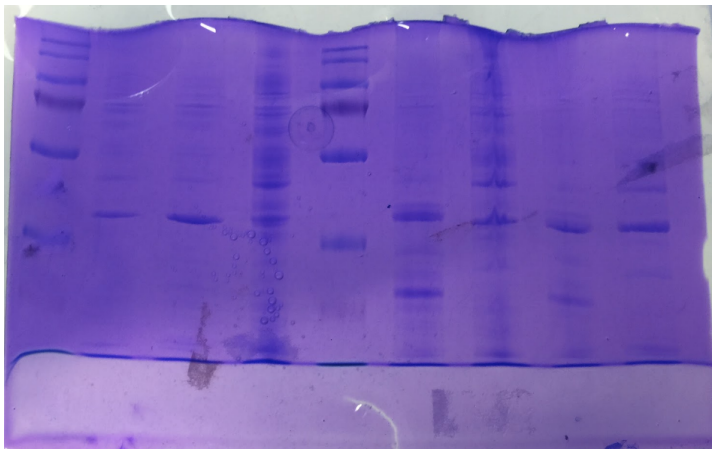

Figure 1: Result of the 12% SDS page. (1) Protein Ladder (2) Soluble proteins from EB(A) BL21 cells after 4h, 1 mM IPTG induction (3) Soluble proteins from EB(B) BL21 cells after 4h, 1 mM IPTG induction (4) Soluble proteins from EB(B) BL21 cells without IPTG induction (5) Protein Ladder 4 (6) Insoluble proteins from EB(A) BL21 cells after 4h, 1 mM IPTG induction (7) Insoluble proteins from EB(A) BL21 cells without induction (8) Insoluble proteins from EB(A) BL21 cells without induction (9) Insoluble proteins from EB(B) BL21 cells after 4h, 1 mM IPTG induction (10) Insoluble proteins from EB(B) BL21 cells without induction.

An intense band at around 40 kDa exists in all the protein samples. Insoluble protein at around 29 kDa showed a significant increase after IPTG induction.

Discussion and Troubleshooting

The expression of pSB1K3-T7-EB seems to be successful. EB is calculated to be 30 kDa and we observed an intense band around 30 kDa after IPTG induction. However, Western blot might be needed to further confirm the result. As Coomassie blue staining is not specific to EB. Besides, the protein ladder used in this SDS page does not have a band at 30 kDa. The band observed might not be exactly at 30 kDa.

2 SDS page for pSB1K3-T7-EB expression second attempt

Responsible

Shuangjia Xue, Oscar Frisell and Reskandi Rudjito

Protocols used

SDS page

Modifications and comments to protocols

The same as the previous one.

Results and Conclusions

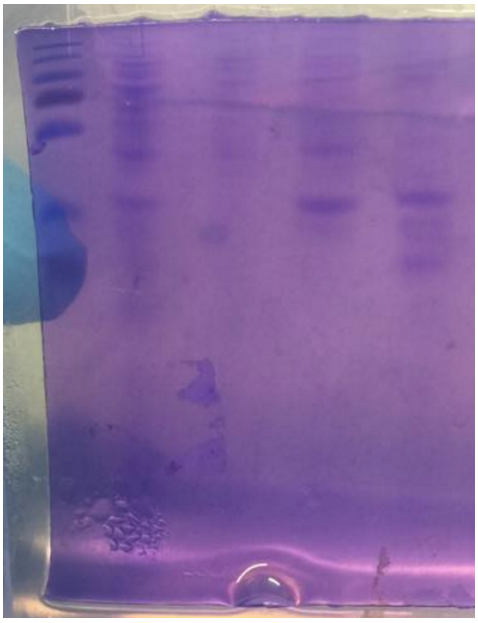

Figure 2: Result of the 12% SDS page. (1) Protein Ladder (2) Insoluble proteins from EB BL21 cells after 4h, 1 mM IPTG induction (3) Insoluble proteins from EB BL21 cells without induction (4) Soluble proteins from EB BL21 cells after 4h, 1 mM IPTG induction (5) Soluble proteins from EB BL21 cells without induction.

Discussion and Troubleshooting

It's hard to draw conclusion from this gel. As the size of interest around 30kDa is not well separated. And the staining is not long enough. The quality of SDS is not as good as the previous one.

3 PCR adding BamHI overhang to T7-EB first trial

Responsible

Shuangjia Xue and Oscar Frisell

Protocols used

Taq polymerase PCR protocol

Modifications and comments to protocols

Volume of Taq polymerase	0.125 μl
Total reaction volume	12.5 μl
Primers used	VF-2F and EB-Rev

Experimental Data

Table 1: Set up of each reaction in the first trial

Reagents	Volume [μl]	Final Concentration
10X PCR Buffer	1.25	1X
10mM dNTPs	1	200μM
10μM forward primer	0.625	0.5μM
10μM reverse primer	0.625	0.5μM
Template DNA	1	1ng
Taq Polymerase	0.125	0.05 unit/μl
25mM MgCl2	1.25	2.5mM
Water	adjust to 12.5μl	-

Results and Conclusions

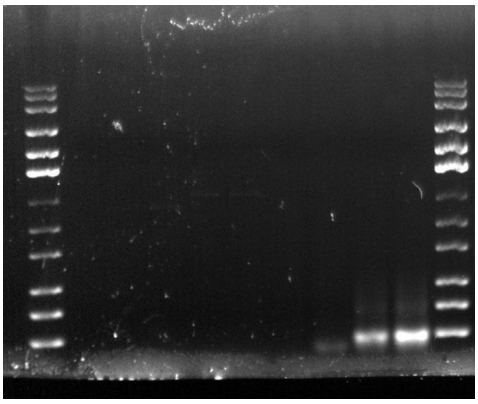

Figure 3: Result of the gel electrophoresis with 1 % agarose (1) DNA Ladder (2-4) irrelevant samples (3) EB1 (4) EB2 (5) Negative Control (6-7)irrelevant samples (8)DNA ladder.

The overhang PCR was unsuccessful as no bands were observed in the gel.

Discussion and Troubleshooting

No bands showed in the gel. The amplification is not successful. The failure might due to unsuitable condition for Taq polymerase or the reagents. The failure could also due to low compatibility of primers. Positive control should be added in the next trial. Increase DNA template or primer amount might also help to improve the results.

4 PCR adding BamHI overhang to T7-EB second trail

Responsible

Shuangjia Xue and Oscar Frisell

Protocols used

Taq polymerase PCR protocol

Modifications and comments to protocols

Similar conditions as the first trial. 1ng, 5ng, 10ng of DNA template and double amount of primers were used to find the optimal conditions for overhang PCR.

Experimental Data

Table 2: Set up of each reaction in the second trial

Reagents	Volume [μl]	Final Concentration
10X PCR Buffer	1.25	1X
10mM dNTPs	1	200μM
10μM forward primer	0.625 or 1.25	0.5 or 1μM
10μM reverse primer	0.625 or 1.25	0.5 or 1μM
Template DNA	1	1, 5 and 10ng
Taq Polymerase	0.125	0.05 unit/μl
25mM MgCl2	1.25	2.5mM
Water	adjust to 12.5μl	-

Results and Conclusions

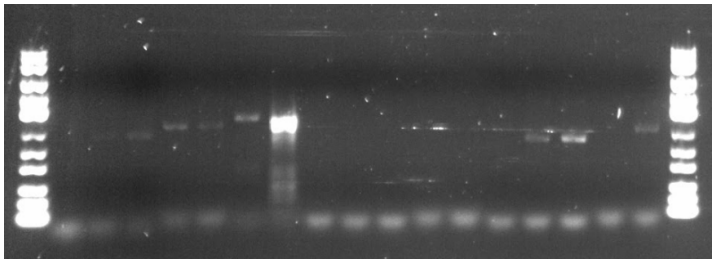

Figure 4: Result of the gel electrophoresis with 1 % agarose (1) DNA Ladder (2-4) irrelevant samples (5) EB1 with 1ng DNA template (6) EB2 with 1 ng DNA template (7) Negative Control (8) positive control (9-11)irrelevant samples (12) EB1 with 1ng DNA and double amount of primers (13) EB2 with 1ng DNA and double amount of primers (14-16) irrelevant samples (17) EB1 with 5ng DNA (18) EB2 with 5ng DNA (19) DNA ladder.

The positive control functions properly but there is band in the negative control due to unknown contamination. We observe faint bands when we use 1ng DNA template, and it seemed to be improved

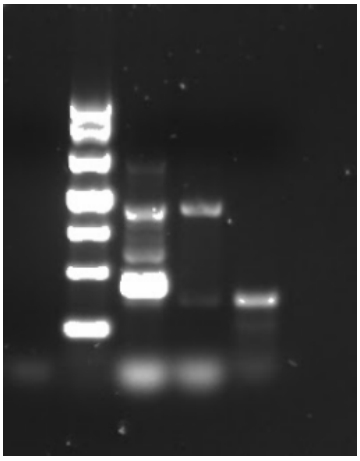

Figure 5: Result of the gel electrophoresis with 1 % agarose (1) DNA Ladder (2) EB1 with 10ng DNA template (3) EB3 with 10ng DNA template (4) EB3 with 10ng DNA template

with the increased DNA template. The bands are more intense in 5ng and 10ng DNA template reactions.

Discussion and Troubleshooting

The overhang PCR seems to work as we increase the DNA template. Though we observe several bands in the 10ng reaction, we decide to cut and purify the gel and continue with the ligation and digestion.

5 Gel purification of EB overhang PCR product

Responsible

Shuangjia Xue and Oscar Frisell

Protocols used

Gel purification

Experimental Data

Run the gel with 10 μl PCR product and 2.5 μl loading dye. Cut and weight the gel. Do the purification following the protocol.

Table 3: Weight of the gel.

Samples	Weight
EB1	0.0639g
EB2	0.0544g

Results and Conclusions

The purification is successful. The concentration is 15.7ng/μl and 12.4 ng/μl for EB1 and EB2.

6 Digestion and ligation of T7-EB with linker-tag

Responsible

Shuangjia Xue and Oscar Frisell

Protocols used

3A Assembly (Digestion and Ligation)

Modifications and comments to protocols

Digestion is performed with BamHI restriction enzyme. No BSA is needed as the buffer provided is optimal for BamHI reaction. The total volume is 25 μl for digestion.

Experimental Data

Table 4: Set up of each digestion reaction

Reagents	Volume [μl]	Final Concentration
10X Buffer	2.5	1X
EB1 PCR product	6.37	100ng
EB2 PCR product	8.06	100ng
BamHI	1	
Water	adjust to 25μl	-

Run the gel with 10 μl PCR product and 2.5 μl loading dye. Cut and weight the gel. Do the purification following the protocol.

Table 5: Set up of each ligation reaction.

Samples	Volume [μl]
EB	25
Linker-tag	1.2
T4 Ligase	2.5
10X Buffer	3.19

Results and Conclusions

No gel is available. No bands for both ligation and digestion production. The digestiona and ligation are not successful.

Discussion and Troubleshooting

The failure of digestion and ligation could due to several reasons. The conditions for the reaction is not optimal. And the PCR purified product does not show any bands, there might be problems with the overhang PCR or gel purification.